Triggering of Methane Production in Rice Soils by Root Exudates: Effects of Soil Properties and Crop Management

S. Mitra, M. S. Aulakh, R. Wassmann, and D. C. Olk*

ABSTRACT

Elevated CH_4 production in flooded soils during the reproductive growth stages of lowland rice (*Oryza sativa* L.) is believed to result from decomposition of root exudates and autolysed root tissue. Little else is known about the factors of late-season CH_4 production. This laboratory study investigates the effects of soil properties and crop management practices on CH_4 production from rice root exudates. In two anaerobic incubation experiments of 20-d duration, CH_4 production was measured following addition of root exudates and glucose to Philippine rice soils that were collected from (Exp. I) five farmers' fields and (Exp. II) four field treatments that varied in degree of soil aeration through crop rotation and timing of crop residue incorporation. The conversion of glucose-C to CH_4 was 1.6 to 3.6 times greater than the conversion of root exudate C. In Exp. I, rates of CH_4 production differed among the five rice soils. The sole soil property that was correlated with cumulative CH_4 production was cation-exchange capacity (CEC). In Exp. II, however, no soil property was correlated with CH_4 production from glucose and root exudates. Instead, CH_4 production was greatest in the soils that had been sampled from the better-aerated field treatments, which opposes the common association of CH_4 production with anaerobic conditions. The exact reason for this trend is unknown. One possible explanation is that organic matter in soils of the better-aerated field treatments provided less chemical stabilization of the amended substances, enabling their faster conversion into CH_4. Soil properties alone appear inadequate to explain differences in CH_4 production from root exudates; crop management practices appear to play a role.

Irrigated lowland rice fields are one of the most important CH_4 sources worldwide. Estimated annual CH_4 emission from rice fields ranges from 57 to 82 Tg yr^{-1} (Bachelet and Neue, 1993) and may contribute 10 to 15% of global CH_4 emissions (Neue, 1993). Rice plants play an important role in regulating the CH_4 budget of rice fields (Rennenberg et al., 1992; Neue and Sass, 1998; Nakićenović et al., 2000). They provide methanogenic substrate through crop residues, root exudates and decaying root tissues, they transport CH_4 and O_2 between soil and atmosphere through a well-developed system of intercellular air spaces (aerenchyma), and they create an active CH_4–oxidizing site in the rhizosphere (Mitra et al., 1999; Wassmann and Aulakh, 2000).

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Methane formation during the early and mid-season growth stages of lowland rice, which last 40 to 50 d in tropical climates, results primarily from microbial decomposition of freshly incorporated crop residues (Wassmann et al., 2000; Chidthaisong and Watanabe, 1997). By contrast, high CH_4 fluxes during the late-season reproductive period of rice have been attributed to microbial decomposition of rice root exudates and autolysed root tissues (Lindau et al., 1991; Sass and Fisher, 1997), because they occur solely in the presence of growing plants and at a crop growth stage marked by high root activity (Holzapfel-Pschorn et al., 1986; Schütz et al., 1989a). Direct evidence for CH_4 formation from root exudates was obtained through pulse labeling of rice plants with $^{14}CO_2$ (Dannenberg and Conrad, 1999), and the influence of root exudates and other exogenous substances on CH_4 production under controlled conditions has been studied for some soils (Wassmann et al., 1998; Lu et al., 2000; Aulakh et al., 2001b). Yet, additional information is desirable on CH_4 production from root exudates for a wider range of soil conditions.

Management practices such as rotation with upland crops, timing of fertilizer application relative to crop residue decomposition, and soil aeration at time of crop residue incorporation can affect several properties of irrigated lowland rice soils, including amount and quality of soil organic matter, microbial biomass, and N supply (Witt et al., 1998). These management practices are known to alter CH_4 formation from decomposing crop residues during the early and mid-season stages of rice crop growth (Abao et al., 2000). Yet limited information exists whether management-induced changes in soil properties also affect CH_4 formation during reproductive growth stages.

In this study, root exudates and glucose were added under controlled conditions to several lowland rice soils and the resulting triggering of methane production was measured. To distinguish the influences of intrinsic soil properties on CH_4 production from those of crop management practices, root exudates and glucose were added to two sets of lowland rice soils from the Philippines: (i) five farmers' fields of double-cropped rice that differed in intrinsic soil properties (Exp. I); and (ii) four crop management treatments that were part of three field experiments on the same soil body (Exp. II). The four treatments varied in removal of aboveground crop residue, crop rotation, and timing of crop residue incorporation relative to soil flooding.

Abbreviations: CEC, cation exchange capacity; IRRI, International Rice Research Institute.

MATERIALS AND METHODS

Collection and Analysis of Soils

In Exp. I (triggering of CH_4 production by root exudate and glucose amendment to different soils), five soils (Bugallon, Luisiana, Famy, Maligaya, and Pila) were sampled in farmers' fields that were located in Laguna Province (Philippines) across an area of about 15-km radius (Table 1). All fields had been in the conventional rotation of double-cropped lowland rice, where rice was grown in flooded soil during both the dry season (January–April) and wet season (July–October). Under this rotation fields are normally drained shortly before the harvest of each crop, allowing the surface soil to air-dry during the subsequent 2-mo fallow. The fields of Exp. I were managed under standard cultivation practices with recommended rates of mineral fertilizers.

In Exp. II (effect of crop management on the triggering of CH_4 production by root exudates and glucose), the Maahas clay soil was sampled from four treatments within three field experiments on the research farm of the International Rice Research Institute (IRRI), Los Baños, Laguna, Philippines (Table 1). All fields were in annual double-cropped rotations. The IRRI-1 soil was under the conventional rice–rice rotation. During several years before sampling, aboveground crop residues had been removed from this field after the harvest of each crop. Because no tillage was done after harvest, decomposition of the crop roots in the dry surface soil was probably delayed largely until the end of the fallow, when the field was tilled and flooded in preparation for the subsequent crop, following standard management practices. Recommended fertilizer rates were applied to each crop: 120 kg N ha^{-1} (as urea), 26 kg P ha^{-1} (as superphosphate), and 50 K kg ha^{-1} (as KCl). The IRRI-2 soil was under a rice–rice rotation during the previous 5 yr, and aboveground crop residues were returned to the soil during tillage at the end of the fallow. This "late" incorporation was about 18 d before transplanting of the subsequent crop and resulted in anaerobic decomposition of all crop residues, the conventional practice of tropical lowland rice farmers including those of Exp. I. Fertilizer rates were the same as for IRRI-1. The IRRI-3 soil was also under a rice–rice rotation. For 5 yr previous to sampling, crop residues were incorporated within 2 to 3 wk after harvest ("early" incorporation), hence decomposing under aerobic conditions that lasted throughout most of the fallows. In this field, the bulk of the crop residues were decomposed within 60 d of early incorporation (Witt et al., 2000). The IRRI-4 soil was from the same field as IRRI-3 but was under a maize (*Zea mays* L.)–rice rotation, where maize was grown in aerated soil during the dry season followed by rice grown in flooded soil during the wet season. During the previous 5 yr, maize and rice residues were incorporated via early incorporation. In the IRRI-3 and IRRI-4 treatments, application of N fertilizer was omitted but each rice crop received 26 kg P ha^{-1} and 50 kg K ha^{-1}.

For all nine field treatments, soil was sampled from the 0- to 15-cm surface layer after harvest of the wet season rice crop in October 1998. Samples were collected with a core sampler of 8-cm diam. For each farmer's field, three corings were taken near the middle of the field and mixed together to make one composite. Each IRRI soil was a composite of soil taken from three replicate plots. Each composite soil was mixed, air-dried, and sieved through a 2-mm screen, and a representative subsample was drawn for all further analyses. The subsamples were stored in darkness at 25°C until the incubation experiments were performed. Three replicates of each composite were used for all analyses.

The soils were analyzed for total C (automated combustion analyzer), $NaHCO_3$–extractable P (Olsen et al., 1954), ammonium acetate-extractable K (Great Britain Ministry of Agriculture, Fisheries, and Food, 1981), dithionite-extractable active Fe and Mn (Asami and Kumada, 1959), CEC (Great Britain Ministry of Agriculture, Fisheries, and Food, 1981), pH in 1:1 soil/water slurries (Peech, 1965), and particle size (Day, 1965).

Substrate Preparation

Thirty rice plants of the high-yielding semidwarf cultivar IR72 were grown in the greenhouse in flooded potted soil as described by Aulakh et al. (2001a). Root exudates were collected at the panicle initiation growth stage following the procedure of Aulakh et al. (2001a, 2001b). Briefly, intact plants were removed from the potted soil and the root system of each plant was placed in a PVC tube containing 500 mL of 0.01 *M* $CaSO_4 \cdot 2H_2O$. This solution provided the root cells with an osmotic environment that approximated the soil solution, enabling more realistic rates of root exudation compared with a deionized water medium (Aulakh et al., 2001a). It also enabled subsequent analyses for total solution C and organic acids; nutrient solutions, while more representative of the soil solution, interfered with these analyses. After 2 h, the $CaSO_4$ solution was removed, and the combined exudate solution from all plants (15 L) was filtered successively through Whatman No. 42 filter paper and a 0.45-μm membrane filter to remove root detritus and microbial cell debris. Then it was concentrated to 600 mL through freeze-drying. This final solution of root exudates was added to the soils in both Exp. I and II, and it had a concentration of 1.2 g total organic C L^{-1} as determined through the wet digestion procedure described by Nelson and Sommers (1996) and modified by Aulakh et al. (2001a). Individual organic acids in the exudate solution

Table 1. Selected properties of the soils used in Experiments I and II.

Soil	Philippine province	Soil order (USDA)	Total C	C/N ratio	Active Fe	Active Mn	pH	Available P	Available K	CEC	Clay	Silt
			g kg^{-1}		g kg^{-1}		1:1 soil/H_2O	mg kg^{-1}	$cmol_c$ kg^{-1}		g kg^{-1}	
					Exp. I							
Bugallon	Pangasinan	Typic Haplaquoll	24.3	10.6	6.5	0.2	6.9	3.4	0.1	56.8	290	570
Luisiana	Laguna	Aquic Troporthent	18.1	10.1	43.3	0.9	4.3	4.1	0.1	24.2	290	660
Famy	Laguna	Vertic Tropaquept	14.9	11.5	12.6	0.5	6.1	11.0	0.2	18.7	420	540
Maligaya	Nueva Ecija	Ustic Endoquerts	11.8	14.8	16.7	1.5	6.2	1.8	0.3	31.1	690	260
Pila	Laguna	Andaqueptic Haplaquoll	45.5	12.6	11.3	1.6	7.5	62.0	0.4	44.2	210	580
					Exp. II							
IRRI-1	Laguna	Aquandic Epiaquoll	12.4	–	17.3	0.7	6.7	9.4	0.6	38.2	520	360
IRRI-2	Laguna	Aquandic Epiaquoll	16.4	–	20.5	0.7	6.4	15.0	0.9	37.7	550	360
IRRI-3	Laguna	Aquandic Epiaquoll	16.0	11.4	25.7	0.9	6.8	32.0	1.1	37.6	580	390
IRRI-4	Laguna	Aquandic Epiaquoll	14.2	11.7	27.4	1.3	6.9	29.0	1.1	37.2	580	360

were measured by the protocol of Badoud and Pratz (1986) as modified by Aulakh et al. (2001a). Total carbohydrate content of the exudate solution was determined by the anthrone colorimetric assay (Brink et al., 1960).

In both Exp. I and II, glucose was considered the standard source of exogenous organic C for CH_4 production, as it has been used in previous studies (Lu et al., 2000). A stock glucose solution of concentration 3.47 m*M* was diluted to the same C concentration as the final root exudate solution (1.2 g total organic C L^{-1}). The glucose stock solution and the initial root exudate solution were diluted with 0.025 *M* KH_2PO_4–Na_2HPO_4 buffer solution so that both final solutions had a pH of 6.8.

Measurement of Methane Production

For both experiments, 81 incubation vessels (nine soils × three substrate treatments × three laboratory replications) were prepared by mixing 20 g of air-dried soil with 20 mL of deionized water in 100-mL spoutless glass beakers, each containing a magnetic bar. Each beaker was sealed with a rubber stopper equipped with a gas outlet and an inlet as described by Mitra et al. (2002a). The soil suspensions were de-aerated with N_2 gas for 3 min at a flow rate of 300 mL min^{-1} while the suspensions were stirred simultaneously. Then the vessels were capped and pre-incubated for 70 d at 30°C, by which time the soils had attained their equilibrium redox potential (E_H) values. This length of preincubation ensured the reestablishment of anaerobic conditions following the air-drying and grinding of the soils so that subsequent addition of C substrates and ensuing methanogenesis would occur under conditions representative of late-season crop growth stages. Headspace air was periodically sampled during this period to measure the background CH_4 production rate before spiking with the root exudate or glucose solution.

After 70 d, substrate treatments (glucose, root exudates, and unamended control) were imposed by adding 20 mL of either deionized water (control) or the final solutions of glucose or root exudates into an incubation vessel, resulting in a final soil/water ratio of 1:2. Immediately after spiking, the soil suspensions were de-aerated with N_2 gas for 3 min and reincubated at 30°C. For both Exp. I and II, daily CH_4 production was measured 2 h, 6 h, 12 h, 1 d, 2 d, 3 d, 7 d, 13 d, and 20 d after spiking. Twenty-four hours before an incubation vessel would be sampled for CH_4 production, it was flushed with N_2 gas at 250 mL min^{-1} for 3 min to remove accumulated CH_4. At each sampling time, triplicate 1-mL gas samples of the headspace of each vessel were removed through a septum for determination of CH_4. Further details on the sampling procedure were provided by Mitra et al. (2002a).

Methane concentration was determined on a gas chromatograph equipped with a flame ionization detector and a Porapak N column (100/200 mesh, 2 m × 20 mm i.d.), with N_2 as the carrier gas. The column and detector temperatures were 60 and 150°C, respectively. The CH_4 production rate was computed using the following equation:

$$\text{Methane production rate (g CH}_4\ \text{g}^{-1}\ \text{soil d}^{-1}) = (dc/dt)\ [(V_{hs} \times MW)/(W_s \times MV)] \quad [1]$$

where dc/dt represents the change in the concentration of CH_4 in the vessel headspace per day (L L^{-1} d^{-1}), V_{hs} the volume of headspace (L), MW the molecular weight of CH_4 (16 g mol^{-1}), W_s the dry weight of soil (g), and MV the molecular volume of CH_4 at 30°C (24.88 L mol^{-1}).

RESULTS AND DISCUSSION

Soil Properties

Total soil C content varied four-fold among the nine soils used in Exp. I and II, and active Fe and Mn varied six- and eight-fold, respectively (Table 1). Soil pH values were mildly acidic except for the strongly acidic Luisiana and mildly alkaline Pila. Most on-farm soils (Exp. I) were relatively low in available P and K. By comparison, the IRRI soils (Exp. II) had high levels of available P and K. Silt was the major soil size separate in four of the five on-farm soils, while clay dominated the IRRI soils.

Experiment I: Triggering of CH_4 Production by Root Exudate and Glucose Amendment to Different Soils

By the end of the 70-d pre-incubation, CH_4 production had ceased in the control treatment for the five on-farm soils (Fig. 1a), indicating depletion of all substrates that had initially been available to methanogens. For all five soils, the total amount of CH_4 produced during this pre-incubation was similar between the unamended control vessels and those incubation vessels that were dedicated to subsequent addition of glucose or root exudates (Fig. 2a).

After the 70-d pre-incubation, the rate of CH_4 production began increasing in all soils within hours after spiking of glucose (Fig. 1b) or root exudates (Fig. 1c), confirming the finding of Aulakh et al. (2001b) that root exudates can contribute to CH_4 production. During the next 8 d, the rate of CH_4 production in all soils remained faster following glucose addition than following root exudate addition. The rate of CH_4 production varied considerably among the five soils; for example after 24 h it varied by two- to four-fold. During the first 4 d the Bugallon soil had the highest production rate for both the glucose and root exudates treatments, and Famy and Pila had among the lowest rates.

This increased production of CH_4 was transient; within 12 d of spiking the daily rate of CH_4 production had decreased considerably. The pattern of decrease varied considerably among soils; the decrease began soonest and was steepest for the Bugallon soil in the glucose treatment, and the CH_4 production rate decreased faster in the glucose treatment than in the root exudate treatment for all soils.

Cumulative CH_4 production during the 20-d incubation of the glucose treatment was at least double that of the root exudate treatment in four of the five soils (Fig. 2b). Total methane production in the Bugallon soil was double that in the Famy soil for both glucose and root exudate treatments, and the other three soils had intermediate levels of production.

Because all five soils had relatively similar histories of land use and crop management, the variation in pattern and amount of CH_4 production suggests that the stimulating effect of root exudates on CH_4 production is influenced by intrinsic soil properties, as has been shown previously for CH_4 production from incorporated crop residues (Wassmann et al., 1998). Of the soil prop-

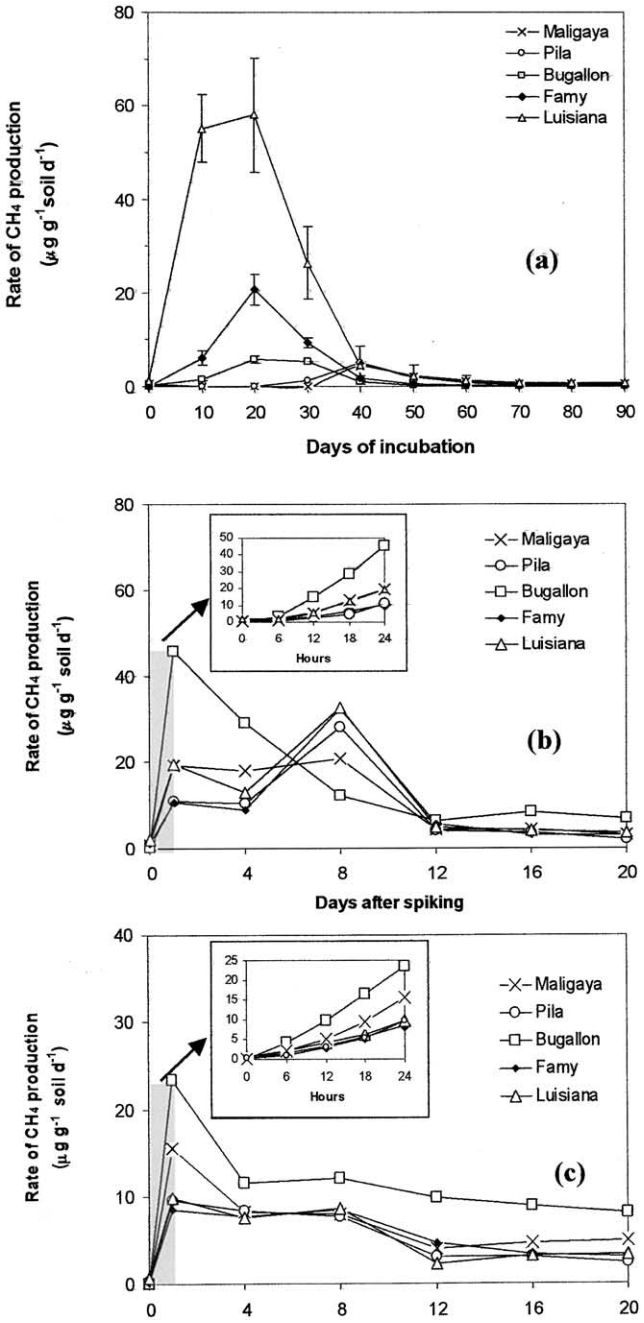

Fig. 1. Daily rates of CH_4 production for lowland rice soils taken from five farmers' fields for (a) the unamended control treatment during a 70-d pre-incubation plus an additional 20 d that corresponded to the post-spiking incubation, and during a 20-d incubation following spiking at 70 d with (b) glucose and (c) rice root exudates. From 0 to 70 d, trends in the control treatment were identical to those of the glucose and root exudate treatments (not shown). Vertical bars represent standard errors of three laboratory replicates. Units of the samplings from 0 to 24 h after spiking (inset graphs) are μg CH_4 g^{-1} soil d^{-1}.

erties listed in Table 1, however, only CEC was significantly correlated ($P < 0.05$) with cumulative CH_4 production, and then only for the root exudate treatment. This association was positive, consistent with the results of Mitra et al. (2002a, 2002b) for CH_4 production from unamended soil.

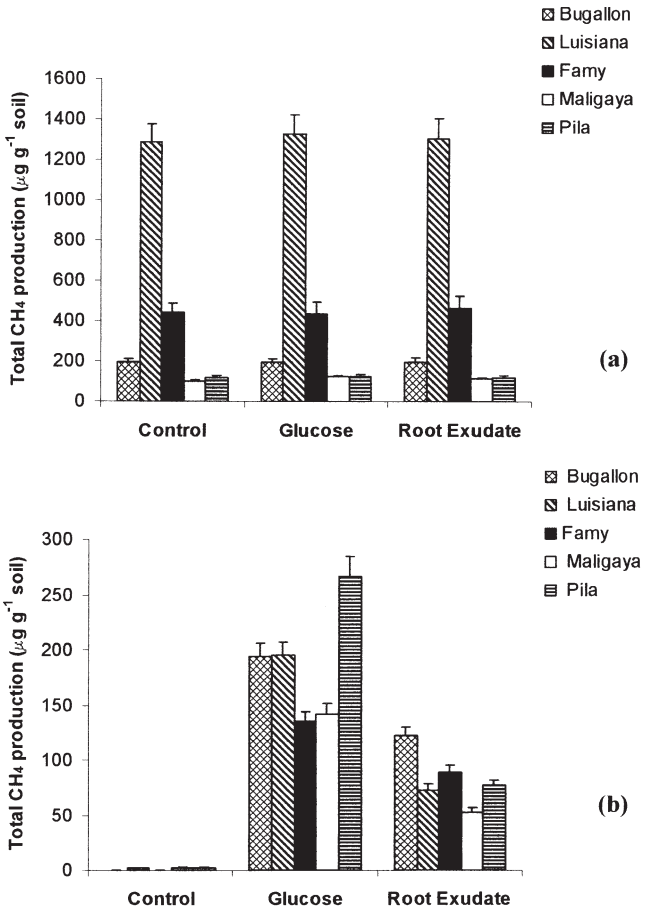

Fig. 2. Total CH_4 production for five rice soils taken from farmers' fields (a) during a 70-d pre-incubation, and (b) during a 20-d incubation following spiking with glucose or root exudates compared with the unamended control treatment. Vertical bars represent standard errors of three laboratory replicates.

Experiment II: Effect of Crop Management on the Triggering of CH_4 Production by Root Exudates and Glucose

During the 70-d pre-incubation period, the rate of CH_4 formation in the unamended control treatment remained negligible in the IRRI-1 and IRRI-2 soils and rose only slightly in IRRI-3 and IRRI-4 (Fig. 3). Cumulative CH_4 production during this period differed little between the control treatment and those samples designated for amendment at 70 d with glucose or root exudates (Fig. 4a).

Following spiking with glucose or root exudates, the rate of CH_4 production increased in soils from all four field treatments, but in a manner that appeared to depend on crop management practices (Fig. 3). For IRRI-1 and IRRI-2, the rice–rice treatments with anaerobic decomposition of roots or of all crop residues, the production increase was subdued, with a delayed response following spiking and peak production reached at modest levels after about 16 d. For IRRI-3 and IRRI-4, the rice–rice and rice–maize treatments, respectively, with aerobic decomposition of crop residues, the onset of CH_4 production was more vigorous. Its rate began increasing sharply within hours after spiking with either

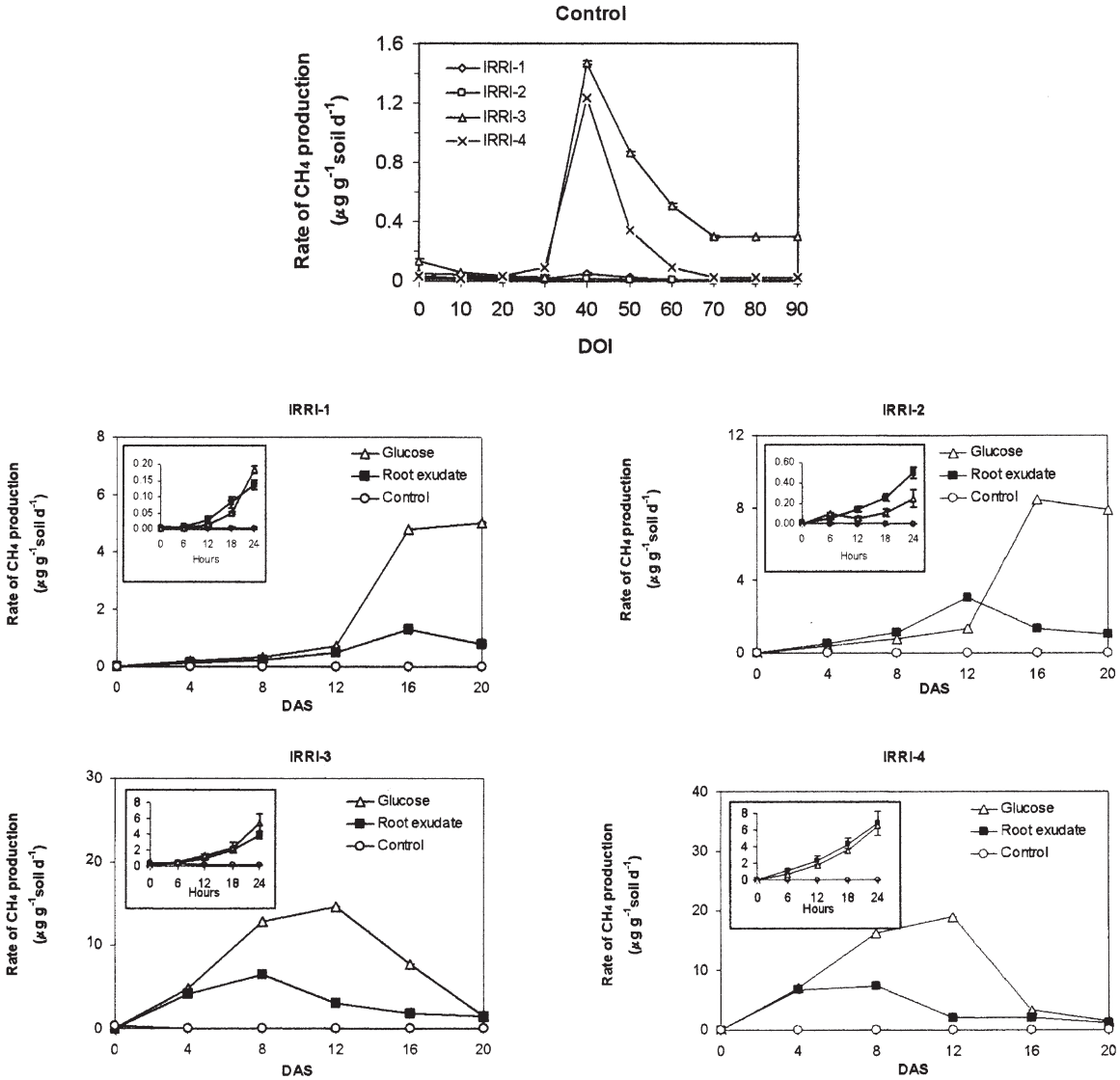

Fig. 3. Daily rates of CH_4 production for lowland rice soils taken from four crop management treatments on the IRRI research farm. IRRI-1 is double-cropped rice with removal of aboveground crop residues and anaerobic decomposition of crop roots. IRRI-2 is double-cropped rice with anaerobic decomposition of all crop residues. IRRI-3 is double-cropped rice with aerobic decomposition of all crop residues. IRRI-4 is a rice-maize rotation with aerobic decomposition of all crop residues. The graph for the unamended control treatment shows production rates during a 70-d pre-incubation plus an additional 20 d that corresponded to the post-spiking incubation. Graphs for each of the four management treatments depict CH_4 production during the 20-d incubation following spiking with glucose or root exudates compared to the unamended control. Units of the samplings from 0 to 24 h after spiking (inset graphs) are μg CH_4 g^{-1} soil d^{-1}. Vertical bars represent standard errors of three laboratory replicates. Abbreviations: DOI, days of incubation; DAS, days after spiking.

root exudates or glucose, and peak production was reached after 8 d (root exudates) or 12 d (glucose) at rates well above those achieved in IRRI-1 and IRRI-2. For the remainder of the 20-d incubation, CH_4 production decreased by a larger amount for IRRI-3 and IRRI-4 than for IRRI-1 and IRRI-2. Cumulative CH_4 production during the 20-d period for both the root exudate and glucose treatments followed the order IRRI-1 < IRRI-2 < IRRI-3 ≤ IRRI-4 (Fig. 4b), although statistical significance cannot be established due to lack of field replication. Methane production in the unamended control vessels was negligible for soils from all four management treatments during this 20-d period (Fig. 3, 4b), indicating that CH_4 production after spiking in these four soils reflected differences in the conversion of the amended glucose and root exudates to CH_4.

In soils from all four field treatments, cumulative CH_4 production during the 20-d incubation for the glucose treatment was 2.9- to 3.6-fold that for root exudates. Similarly in Exp. I, the range was 1.6- to 2.7-fold, although glucose and root exudates were added at the same rate of 1.2 g C kg^{-1} soil in both experiments. In previous studies, glucose amendment enhanced CH_4 production compared with acetate amendment in two wetland soils from the Florida Everglades (Bachoon and Jones, 1992) and two Philippine rice soils (Lu et al., 2000).

The consistently higher production of CH_4 from glu-

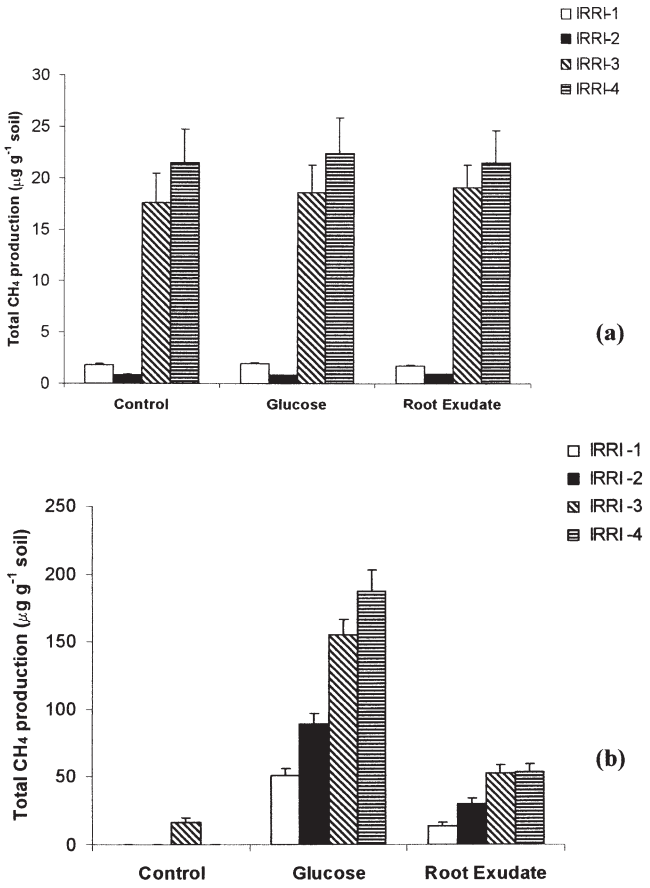

Fig. 4. Total CH_4 production for rice soils taken from four crop management treatments on the IRRI farm (a) during a 70-d pre-incubation, and (b) during a 20-d incubation following spiking with glucose or root exudates compared with the unamended control treatment. Management treatments are explained in Fig. 3. Vertical bars represent standard errors of three laboratory replicates.

cose than from root exudates during the 20-d incubation is likely due to substrate quality. Glucose contains easily degradable C, whereas root exudates contain a variety of C compounds, including organic acids such as malic, tartaric, succinic, citric, and lactic acid (Table 2). Compared with glucose, simple organic acids have been observed to better resist microbial degradation under flooded conditions (Tate, 1979; Schütz et al., 1989b). A second possible cause of the greater CH_4 production in the glucose treatment was the high (approximately 0.12 *M*) sulfate concentration in the solution of the soils spiked with root exudates. Sulfate levels greater than 60 to 105 μM can stimulate sulfate reducers, which suppress methanogens through substrate competition (Lovley and Klug, 1983). Lu et al. (2000), however, collected root exudates in a sulfate-free solution and still found they provided less CH_4 production than did glucose when added to some of the same soils used in this study, suggesting an intrinsic difference between root exudates and glucose in their potential for CH_4 production.

Table 2. Composition of the final root exudate solution that was added to the soil samples after 70-d pre-incubation. The root exudates were collected at the panicle initiation growth stage of the rice plants.

Compound	Concentration
Malic acid	12.06 m*M*
Tartaric acid	1.21 m*M*
Succinic acid	Not detected
Citric acid	1.74 m*M*
Lactic acid	Not detected
Total organic acids	0.75 g C L^{-1}
Total carbohydrates	0.45 g C L^{-1}
Total organic C	1.20 g C L^{-1}

The degree to which root exudates and glucose were converted to CH_4 was positively associated in Exp. II with the degree of soil aeration during previous crop management. The greatest CH_4 production during the 20-d incubation occurred in soils from the treatments with aerobic decomposition of either rice residues (IRRI-3 soil) or maize and rice residues (IRRI-4 soil), although statistical significance cannot be established. These results are not consistent with our current understanding of the soil conditions that promote methanogenesis. Specifically, they oppose the general association of CH_4 production with anaerobic soil conditions. They contradict trends in microbial subpopulations of the IRRI soils as determined by fatty acid methyl ester analysis: more anaerobic subpopulations were found in field treatments with (i) anaerobic decomposition of crop residues compared with aerobic decomposition, and (ii) the rice–rice rotation compared with the rice–maize rotation (K. M. Scow, personal communication). Our findings are contrary to the association by Chidthaisong et al. (1999) of increased CH_4 production with application of N fertilizer, as N fertilizer was applied solely to IRRI-1 and IRRI-2. Commonly measured soil properties (Table 1) did not indicate an explanation for CH_4 trends in Exp. II. Although CEC was positively associated with CH_4 production from root exudates in Exp. I, it scarcely varied among the four IRRI soils despite their distinctly different rates of CH_4 production. Active Fe, commonly thought of as a CH_4 suppressant (Ito et al., 2002; Gaunt et al., 1997) was more abundant in IRRI-3 and IRRI-4 than in IRRI-1 and IRRI-2. The soil C/N ratio varied from 10.1 to 14.8 among the soils of Exp. I and had no association with CH_4 production. Total soil N was not available for IRRI-1 and IRRI-2, but the C/N ratios for IRRI-3 and IRRI-4 do not suggest that CH_4 production was associated with more labile soil organic matter, that is, a low C/N ratio.

Amendment of organic compounds accelerated the decomposition of native soil organic matter into CH_4 in other studies (Dalenberg and Jager, 1989; Lu et al., 2000), but we did not observe this effect. Transformation efficiencies, calculated as the molar ratios of methane-C produced/substrate-C amended (Lu et al., 2000), indicated that the quantity of CH_4–C evolved in this study was at most 28% of amended C. Accelerated decomposition of native organic matter would be indicated only when this value exceeds the 50% efficiency that occurs with stoichiometric conversion of glucose to CH_4 (Lu et al., 2000).

Hence the positive association of soil aeration with CH_4 production from root exudates and glucose must involve other processes that have not yet been associated with methanogenesis. Root exudates and cellular remnants of microorganisms that fed off the root exu-

dates and glucose were likely incorporated to some degree into the soil organic matter, which involves chemical binding. As one possible explanation for our results, we speculate that young soil organic matter in soils from the better aerated treatments (IRRI-3 and IRRI-4) formed fewer or weaker chemical bonds with the root exudates, glucose, and cellular remnants than did the young organic matter that formed under the more anaerobic conditions of IRRI-1 and IRRI-2. Consequently more root exudate-C or glucose-C remained available to methanogens in IRRI-3 and IRRI-4 for short-term CH_4 production.

This speculation is based on the results of Olk and Cassman (2002), who studied N cycling in the field experiment that was sampled for the IRRI-3 and IRRI-4 soils. For the double-cropped rice rotation, in-season mineralization from young organic matter of both total organic N and immobilized ^{15}N fertilizer was greater with aerobic decomposition of crop residues than with anaerobic decomposition. Similar benefits were provided by the maize–rice rotation compared with double-cropped rice. Simultaneously, an accumulation of phenolic compounds was associated with the inhibited N mineralization under anaerobic decomposition of crop residues and under double-cropped rice. Analysis by nuclear magnetic resonance spectroscopy provided direct evidence for covalent binding of soil organic N by phenolic lignin residues in a triple-cropped rice soil where anaerobic decomposition was practiced (Schmidt-Rohr et al., 2004). Anaerobic decomposition of plant materials under controlled conditions promoted chemical stabilization of organic (Chen et al., 1993) and inorganic (Ceccanti and Ding, 1985; Noguchi et al., 1997) compounds, and phenolic compounds commonly accumulate in anaerobic soils (Malcolm, 1990).

Hence phenolic-rich organic matter in soils from the anaerobic treatments of Exp. II could conceivably stabilize more strongly the root exudates and microbial remnants that were formed from the root exudates and glucose. Such stabilization would be consistent with the observed effects of previous management practices on CH_4 production from root exudates despite the lack of perceptible differences in commonly measured soil properties. Besides soil aeration there are likely additional factors that control CH_4 production from root exudates, as soil aeration cannot explain the generally higher rates of CH_4 production in the soils of Exp. I that had been under more anaerobic field conditions compared with the IRRI-3 and IRRI-4 soils of Exp. II.

CONCLUSIONS

Decomposition of root exudates in submerged soils led to CH_4 production, although at a slower rate than following glucose amendment. Rates of CH_4 production varied among soils, but they were not closely related to any intrinsic soil property for both experiments. In Exp. II, CH_4 production was positively associated with the degree of soil aeration in the field treatments sampled for this experiment. Aerobic soil conditions had been promoted by rotation with upland maize or by aerobic decomposition of crop residues. We speculate that soil aeration changed the chemical nature of young soil organic matter such that it provided less chemical stabilization of the amended substances and enabled their faster conversion into CH_4, compared with the more anaerobic treatments.

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REFERENCES

Abao, E.B., Jr., K.F. Bronson, R. Wassmann, and U. Singh. 2000. Simultaneous records of methane and nitrous oxide emissions in rice-based cropping systems under rainfed conditions. Nutr. Cycling Agroecosyst. 58:131–139.

Asami, T., and K. Kumada. 1959. A new method for determining free iron in paddy soils. Soil Plant Food 5:141–146.

Aulakh, M.S., R. Wassmann, C. Bueno, J. Kreuzwieser, and H. Rennenberg. 2001a. Characterization of root exudates at different growth stages of ten rice (*Oryza sativa* L.) cultivars. Plant Biol. 3:139–148.

Aulakh, M.S., R. Wassmann, C. Bueno, and H. Rennenberg. 2001b. Impact of root exudates of different cultivars and plant development stages of rice (*Oryza sativa* L.) on methane production in a paddy soil. Plant Soil 230:77–86.

Bachelet, D., and H.U. Neue. 1993. Methane emissions from wetland rice areas of Asia. Chemosphere 26:219–237.

Bachoon, D., and R.D. Jones. 1992. Potential rates of methanogenesis in sawgrass wetlands with peat and marl soils in the Florida Everglades. Soil Biol. Biochem. 24:21–27.

Badoud, R., and G. Pratz. 1986. Improved high-performance liquid chromatographic analysis of some carboxylic acids in food and beverages as their *p*-nitrobenzyl esters. J. Chromatogr. 360:119–136.

Brink, R.H., Jr., P. Dubach, and D.L. Lynch. 1960. Measurement of carbohydrates in soil hydrolyzates with anthrone. Soil Sci. 89:157–166.

Ceccanti, B., and C.P. Ding. 1985. Anaerobic decomposition of green manures in flooded soils, in the presence of $Cr(VI)^{+2}$ charge and size characterization of decaying products and their metal-complexes. Agrochimica 29:50–65.

Chen, K.-L., H. Itoh, and J. Hayashi. 1993. Change of rice straw lignin with NaOH-oxygen pulping II. Comparison of rice straw and wood lignins upon a non-stirring oxidation with NaOH-oxygen. Mokuzai Gakkaishi 39:459–464.

Chidthaisong, A., H. Obata, and I. Watanabe. 1999. Methane formation and substrate utilization in anaerobic rice soils as affected by fertilization. Soil Biol. Biochem. 31:135–143.

Chidthaisong, A., and I. Watanabe. 1997. Methane formation and emission from flooded rice soil incorporated with ^{13}C-labelled rice straw. Soil Biol. Biochem. 29:1173–1181.

Dalenberg, J.W., and G. Jager. 1989. Priming effect of some organic additions to ^{14}C-labelled soil. Soil Biol. Biochem. 21:443–448.

Dannenberg, S., and R. Conrad. 1999. Effect of rice plants on methane production and rhizospheric metabolism in paddy soil. Biogeochemistry 45:53–71.

Day, P.R. 1965. Particle fractionation and particle-size analysis. p. 545–567. *In* C.A. Black (ed.) Methods of soil analysis. Part I. ASA, Madison, WI.

Gaunt, J.L., H.U. Neue, J. Bragais, I.F. Grant, and K.E. Giller. 1997. Soil characteristics that regulate soil reduction and methane production in wetland rice soils. Soil Sci. Soc. Am. J. 61:1526–1531.

Great Britain Ministry of Agriculture, Fisheries, and Food. 1981. Cation exchange capacity and exchangeable cations in the soil. p.

197–203. *In* The analysis of agricultural materials: A manual of the analytical methods used by the Agricultural Development and Advisory Service. 2nd ed. Her Majesty's Stationery Office, London.
Holzapfel-Pschorn, A., R. Conrad, and W. Seiler. 1986. Effects of vegetation on the emission of methane from submerged paddy soil. Plant Soil 92:223–233.
Ito, T., M. Hanaki, and M. Saigusa. 2002. Effects of iron oxide on methane emission rates and rice growth in paddy soils: A pot experiment. p. 1491–1 to 1491–6. *In* Proceedings, 17th World Congress of Soil Science. 14–21 Aug. 2002, Bangkok, Thailand. International Union of Soil Sciences.
Lindau, C.W., P.K. Bollich, R.D. DeLaune, W.H. Patrick, Jr., and V.J. Law. 1991. Effect of urea fertilizer and environmental factors on CH_4 emissions from a Louisiana, USA rice field. Plant Soil 136:195–203.
Lovley, D.R., and M.J. Klug. 1983. Sulfate reducers can outcompete methanogens at freshwater sulfate concentrations. Appl. Environ. Microbiol. 45:187–192.
Lu, Y., R. Wassmann, H.U. Neue, C. Huang, and C.S. Bueno. 2000. Methanogenic responses to exogenous substrates in anaerobic rice soils. Soil Biol. Biochem. 32:1683–1690.
Malcolm, R.L. 1990. Variations between humic substances isolated from soils, stream waters, and groundwaters as revealed by ^{13}C-NMR spectroscopy. p. 13–35. *In* P. MacCarthy et al. (ed.) Humic substances in soil and crop sciences: Selected readings. ASA, Madison, WI.
Mitra, S., M.C. Jain, S. Kumar, S.K. Bandyopadhyay, and N. Kalra. 1999. Effect of rice cultivars on methane emission. Agric. Ecosyst. Environ. 73:177–183.
Mitra, S., R. Wassmann, M.C. Jain, and H. Pathak. 2002a. Properties of rice soils affecting methane production potentials: 1. Temporal patterns and diagnostic procedures. Nutr. Cycling Agroecosyst. 64:169–182.
Mitra, S., R. Wassmann, M.C. Jain, and H. Pathak. 2002b. Properties of rice soils affecting methane production potentials: 2. Differences in topsoil and subsoil. Nutr. Cycling Agroecosyst. 64:183–191.
Nakićenović, N., O. Davidson, G.Davis, A. Grübler, T. Kram, E. L. La Rovere, B. Metz, T. Morita, W. Pepper, H. Pitcher, A. Sankovski, P. Shukla, R. Swart, R. Watson, and Z. Dadi. 2000. Special report on emissions scenarios: A special report of Working Group III of the Intergovernmental Panel on Climate Change. Cambridge University Press, Cambridge.
Nelson, D.W., and L.E. Sommers. 1996. Total carbon, organic carbon, and organic matter. p. 961–1010. *In* D.L. Sparks et al. (ed.) Methods of Soil Analysis, Part 3. SSSA Book Series no. 5. SSSA, Madison, WI.
Neue, H.U. 1993. Methane emission from rice fields. Bioscience 43:466–474.
Neue, H.U., and R. Sass. 1998. The budget of methane from rice fields. IGACtivities 17:3–11.
Noguchi, A., I. Hasegawa, F. Shinmachi, and J. Yazaki. 1997. Possibility of copper deficiency and impediment in ripening in rice from application of crop residues in submerged soil. p. 799–800. *In* T. Ando et al. (ed.) Plant nutrition for sustainable food production and environment: Proceedings of the XIII International Plant Nutrition Colloquium. Kluwer Academic Publishers, Dordrecht.
Olk, D.C., and K.G. Cassman. 2002. The role of organic matter quality in nitrogen cycling and yield trends in intensively cropped paddy soils. p. 1355–1 to 1355–8. *In* Proceedings, 17th World Congress of Soil Science. 14–21 Aug. 2002, Bangkok, Thailand. International Union of Soil Sciences.
Olsen, S.R., C.V. Cole, F.S. Watanabe, and L.A. Dean. 1954. Estimation of available phosphorus in soils by extraction with sodium bicarbonate. USDA Circular 939.
Peech, M. 1965. Hydrogen-ion acidity. p. 914–926. *In* C.A. Black (ed.) Method of soil analysis. Part 2. Agron. Mongr. No. 9. ASA, Madison, WI.
Rennenberg, H., R. Wassmann, H. Papen, and W. Seiler. 1992. Trace gas emission in rice cultivation. Ecol. Bull. 42:164–173.
Sass, R.L., and F.M. Fisher, Jr. 1997. Methane emissions from rice paddies: A process study summary. Nutr. Cycl. Agroecosyst. 49: 119–127.
Schmidt-Rohr, K., J.-D. Mao, and D.C. Olk. 2004. Nitrogen-bonded aromatics in soil organic matter and their implications for a yield decline in intensive rice cropping. Proc. Nat. Acad. Sci. (U.S.A.) 101:6351–6354.
Schütz, H., A. Holzapfel-Pschorn, R. Conrad, H. Rennenberg, and W. Seiler. 1989a. A 3-year continuous record on the influence of daytime, season, and fertilizer treatment on methane emission rates from an Italian rice paddy. J. Geophysical Res. 94(D13):16,405–16,416.
Schütz, H., W. Seiler, and R. Conrad. 1989b. Processes involved in formation and emission of methane in rice paddies. Biogeochemistry 7:33–53.
Tate, R.L., III. 1979. Effect of flooding on microbial activities in organic soils: Carbon metabolism. Soil Sci. 128:267–273.
Wassmann, R., and M.S. Aulakh. 2000. The role of rice plants in regulating mechanisms of methane emissions. Biol. Fertil. Soils 31:20–29.
Wassmann, R., L.V. Buendia, R.S. Lantin, C.S. Bueno, L.A. Lubigan, A. Umali, N.N. Nocon, A.M. Javellana, and H.U. Neue. 2000. Mechanisms of crop management impact on methane emissions from rice fields in Los Baños, Philippines. Nutr. Cycling Agroecosyst. 58:107–119.
Wassmann, R., H.U. Neue, C. Bueno, R.S. Lantin, M.C.R. Alberto, L.V. Buendia, K. Bronson, H. Papen, and H. Rennenberg. 1998. Methane production capacities of different rice soils derived from inherent and exogenous substrates. Plant Soil 203:227–237.
Witt, C., K.G. Cassman, D.C. Olk, U. Biker, S.P. Liboon, M.I. Samson, and J.C.G. Ottow. 2000. Crop rotation and residue management effects on carbon sequestration, nitrogen cycling and productivity of irrigated rice systems. Plant Soil 225:263–278.
Witt, C., K.G. Cassman, J.C.G. Ottow, and U. Biker. 1998. Soil microbial biomass and nitrogen supply in an irrigated lowland rice soil as affected by crop rotation and residue management. Biol. Fertil. Soils 28:71–80.